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Application for Research Grant
(Use extra pages as needed)

Date July 1, 1975

1. Principal Investigator (give title and degrees):

Gerard M. Turino, M.D.
Professor of Medicine

2. Institution & address:

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630 West 168th Street
New York, New York 10032

3. Department(s) where research will be done or collaboration provided:

Departments of Medicine, Pathology and Obstetrics and Gynecology

4. Short title of study:

Chemical Basis of Tissue Destruction in Obstructive Lung Disease

5. Proposed starting date: October 1, 1975

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

To define the role of elastase activity of alveolar macrophages and of circulating polymorphonuclear leukocytes in the development of experimental pulmonary emphysema in animals and in the etiology of emphysematous destruction of the lung in human subjects.

1. Within this objective, an attempt will be made to determine differences for alveolar macrophage elastase activity between patients who have obstructive airways disease as compared with normal subjects as has already been demonstrated in this Laboratory for the polymorphonuclear leukocytes between these two groups. In addition other distinctions include differences in elastase activity among patients who have primarily pulmonary emphysema as compared with patients who have primarily asthma or asthmatic bronchitis with less pulmonary parenchymal destruction. Also, distinctions will be sought between normal subjects who have a history of smoking as compared with non-smokers.

2. The response of polymorphonuclear leukocyte and alveolar macrophage elastase activity to the following interventions in experimental animals (rats and hamsters) will be quantified: a) prolonged daily exposure to tobacco smoke over a four to six week period and after cessation, b) prolonged exposure to NO_2 at concentrations in inspired air of 15 ppm and NO_2 and O_3 of .9 ppm each (approximately six weeks), c) pulmonary injury induced by intratracheal and intravenous injection of pancreatic elastase and papain intratracheally.

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7. Brief description of specific research aims (continued)

3. To determine the feasibility of using tissue cultures of alveolar macrophages from normal human lungs and from patients with obstructive lung disease for the identification of levels of elastase activity and to investigate interventions which may either increase or decrease alveolar macrophage elastase activity. These interventions include a) exposure to tobacco smoke (daily exposure for several weeks), b) hypoxia (10 to 15% O_2), c) exposure of cells and media to high CO_2 atmosphere (5 to 10%) in air and d) chemical acidosis of the media induced by addition of low molar concentrations of hydrochloric acid.

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8. Brief statement of working hypothesis:

The hypothesis of this study is that destruction of pulmonary parenchyma which is commonly a concomitant of obstructive lung disease in man and is a primary etiological factor in pure forms of pulmonary emphysema in the human population is the result of proteolytic injury to connective tissue components of the lung. Injury to pulmonary elastin is an essential feature of this destructive process and the chemical basis for degradation and disruption of elastin resides in both polymorphonuclear leukocytes which are sequestered in the lung and alveolar macrophages which are normal cellular components of the alveolus. It is hypothesized also that certain environmental factors, such as constituents of tobacco smoke, environmental gases or in vivo tissue factors in the lung, stimulate and perpetuate over-activity of cellular elastase and perhaps other proteases.

9. Details of experimental design and procedures (append extra pages as necessary)

Chemical Basis of Tissue Destruction in Obstructive Lung Disease. (The Role of Alveolar and Leukocyte Elastases in the Etiology of Pulmonary Emphysema).

Introduction and Overall Objectives:

Diseases of the lung which cause obstruction to air flow such as pulmonary emphysema, chronic bronchitis and asthma are major causes of morbidity and death in the adult population. At present, we know little of the chemical basis of the pathogenetic processes which produce destruction and alteration of the architecture of the lung. Yet, it is clear that these structural alterations lead to irreversible changes in lung function and severely limit therapeutic effectiveness in these diseases.

Over the past five years, studies from this Laboratory (1-3) and others (4-6) have demonstrated that alteration of lung elastin by elastase leads to marked increases in pulmonary distensibility and in the morphological development of pulmonary parenchymal destruction consistent with pulmonary emphysema. The administration of collagenase produces changes in the tensile strength of lung parenchymal tissue but does not produce pulmonary emphysema (1,4,6). It also is demonstrable that a single exposure of hamster lung in vivo to elastase by the tracheal route leads to subsequent and progressive damage to the alveoli in the following weeks and months leading to the morphological appearance of pulmonary emphysema (7). These observations in experimental preparations have focussed attention on the crucial role of elastin in pulmonary parenchymal tissue to maintain the normal geometric configuration of the alveolus. The structural characteristics of elastin and the relationship of elastin to other connective tissue components such as collagen and glycosaminoglycans which determine the structural role of elastin are beginning to be investigated (8,9). However, such observations indicate the need to understand the chemical and morphological processes by which the initial and the subsequent injury to elastin and the alveolar septal tissue occurs after exposure to elastases.

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9. Details of experimental design and procedures (continued)

Leukocytes contain both elastolytic (10) and collagenolytic enzymes (11). The leukocyte elastase is inhibited by alpha$_1$-antitrypsin and Janoff has shown that the inhibitory component for these elastases is missing in the serum of individuals with alpha$_1$-antitrypsin deficiency (12). Similarly, Ohlsson has reported (13) that leukocyte elastase as well as a second neutral leukocyte protease formed alpha$_1$-antitrypsin complexes with characteristic electrophoretic mobility and more recently Ohlsson has demonstrated at least two collagenases and three elastases which have neutral pH optima and are released to a great extent from the leukocytes during phagocytosis of immune complexes or bacteria (14). He has also pointed out that the relative affinity of granulocyte collagenase for alpha$_2$-macroglobulin is about ten times as strong for alpha$_1$-antitrypsin. However, alpha$_1$-antitrypsin is the major serum inhibitor of elastase.

Alveolar macrophage elastase was first described in 1971 by Janoff, Rosenberg and Galdston (15). Human alveolar cells collected by lavage, as well as alveolar macrophages from rabbit lungs, were studied. However, unlike the leukocyte elastase, very little work has been done on the alveolar macrophage elastases. A recent paper by Harris et al. (16) indicates that smokers have a statistically significantly greater concentration of elastase in alveolar macrophages than non-smokers. In contrast, the relative concentrations of leukocyte elastase are the same in both populations. In addition, it has been known for some time (17, 18) that macrophage cell density is increased approximately four times in smokers as compared with non-smokers. The elastase isolated from alveolar macrophages resembles the leukocyte elastase in several respects but may differ in its substrate specificity, i.e., the exact site of cleavage in the elastin molecule as well as its susceptibility to various inhibitors. Although both enzymes are inhibited by alpha$_1$-antitrypsin in the serum, the alveolar macrophage elastase is inhibited to a much lesser extent. Also, a substance present in one molar NaCl extracts of minced human lung tissue inhibits leukocyte but not macrophage elastase (19). Mass, Ikeda, Meranze, Weinbaum and Kimbel (20) have been able to induce lesions resembling human emphysema by intratracheal administration of alveolar macrophages as well as leukocyte proteases in dogs. It is also of interest that significantly higher levels of alpha$_1$-antitrypsin inhibitor have been found in the alveolar macrophages of smokers which suggests that chronically increased amounts could be a protective mechanism against proteolysis (21).

In studies done thus far by the principal investigator and collaborators, the elastase activity of the circulating polymorphonuclear leukocytes has been found to be statistically significantly higher in the population of patients with chronic obstructive lung disease with normal MM alpha$_1$-antitrypsin phenotype as compared with a population of adult normal subjects (22).

Since the lung normally has a high content of leukocytes which become sequestered in pulmonary capillaries, the alveolar septal area is particularly susceptible to the elastolytic effects of leukocyte lysosomal enzymes. This leukocyte sequestration has been found to be greatly enhanced during endotoxin shock in dogs and has been used as a technique to produce parenchymal destruction from increased elastolytic activity (23, 24). However, the potentially pathogenetic role of the alveolar macrophages which also contain some level of elastolytic activity and which have been shown capable of inducing morphological changes of emphysema in experimental animals resembling the human disease deserves further exploration. These investigations will consider the following:

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9. Details of experimental design and procedures (continued)

1. The elastolytic activity of the normal alveolar macrophage in experimental animals and normal man as compared with leukocyte elastolytic activity.

2. The capacity of alveolar as well as leukocyte elastolytic enzymes to increase or decrease in response to specific in vivo and environmental influences.

3. The dynamics of changes in elastolytic activity in both alveolar macrophages and leukocytes with respect to the duration of effects causing increases or decreases in elastase activity in both species of cells.

4. Ultimately, to extend these investigations to include exploration of the inhibitors of both alveolar and leukocyte elastases which are present both in serum and in the alveolus and which may also respond to pathogenetic stimuli.

This study then is designed to explore the role of elastolytic enzymes of the circulating polymorphonuclear leukocyte and the alveolar macrophage in the pathogenesis of obstructive lung disease in man and in experimental animals.

Experimental Plan

The experimental plan involves two major parts: 1) the study of animal alveolar macrophages and circulating polymorphonuclear leukocytes and 2) studies in human subjects.

1. Animal Studies

The objective in this portion of the study is to measure the elastase activity of alveolar macrophages as compared with polymorphonuclear leukocytes in three species of animals, namely, hamsters, rats and dogs. Control measurements will be done in these species in normal animals to allow a series of experimental studies to determine the effect of various interventions on the elastase activity of both alveolar macrophages and polymorphonuclear leukocytes. These interventions are as follows:

A) Tobacco Smoke

The effect of daily exposure to tobacco smoke for a period of 6 to 10 weeks in each species to determine the ability of whole tobacco smoke to bring about an increase in alveolar and/or polymorphonuclear leukocyte lysosomal elastase activity. If alveolar macrophage elastase is increased the study will be continued to determine the rate at which alveolar or polymorphonuclear leukocyte elastase decreases to normal after cessation of exposure to tobacco smoke.

Rats and hamsters (in groups of 10 animals each) will undergo daily exposure to unfiltered cigarette smoke by a smoking machine using a "standard" reference cigarette in amounts of 4 cigarettes per day over a short interval of the day. This exposure has been shown in guinea pigs (25) to be associated with an increase in free lung cells (both macrophages and leukocytes) after 4 weeks. Also, after such exposure, histological effects on the goblet cells and epithelium, as well as increased amounts of mucus, have been described (25).

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9. Details of experimental design and procedures (continued)

A) Tobacco Smoke (continued)

These findings in guinea pigs are similar to the response of free lung cells in human smokers as compared with non-smokers (.17, 26).

In later phases of this work, two aspects of smoke exposure in rats will be explored further. 1. The elimination of particulate matter in smoke to determine the effect of the absence of particulates on alveolar macrophage and leukocyte elastase activity and 2. The control of intercurrent pulmonary infection by administration of antibiotics to animals during the period of exposure. In the work of Rylander (25) control of both of these factors resulted in significantly less increase in free lung cells and in the histological evidence of bronchial irritation. It would be of importance to assay concomitant effects of these interventions on alveolar macrophage and leukocyte elastase activity.

B) Proteolytic Enzymes

The effect of an initial proteolytic enzyme injury to the lung on the alveolar and polymorphonuclear leukocyte elastase activity. This portion of the study is being undertaken to examine the mechanism by which a single injury to the lung, in this case proteolytic enzyme injury to the pulmonary parenchyma, results in subsequent and progressive damage to the lung resulting in progression of the pulmonary emphysema in experimental animals after an initial insult. While such a result may occur from mechanical forces, leading to altered connective tissue resistance to tissue stresses, the possibility exists that such initial proteolytic injury may result in subsequent increases in elastase activity of alveolar macrophages or polymorphonuclear leukocytes which then have access to the lung. For these experiments, groups of 20 hamsters, and 10 dogs, will be exposed to papain aerosol intratracheally. Levels of alveolar and polymorphonuclear leukocyte lysosomal elastase activity will be measured in the control state before exposure to the proteolytic enzyme, at 2 days after exposure, and then at weekly intervals over the next 1 month to 6 weeks in each species. If it is determined that elastase activity is increased, measurements will be continued for 2 months at which time the animals will be sacrificed and the lungs examined morphologically and by point counting to quantify the extent of emphysema.

C) Nitrogen dioxide

It has been demonstrated that rats (27) exposed to nitrogen dioxide in the inspired air in amounts of 15 ppm and above for approximately 4 months develop morphological emphysema. The basis of this emphysema is unknown. The potential mechanism for tissue destruction resulting from such exposure is the stimulation of elastase activity in cells which normally carry elastolytic enzymes, such as the alveolar macrophage and circulating polymorphonuclear leukocyte.

Accordingly, rats will be exposed to NO_2 according to the technique of Freeman et al. (28) for a period of approximately 4 months. Alveolar macrophages will be lavaged from these animals at intervals of 3 weeks to determine if there is a significant increase in alveolar macrophage elastase activity as compared with a group of control animals exposed to the same control environment but not exposed to NO_2. Also, circulating polymorphonuclear leukocytes will be harvested from pooled blood samples of 4 rats each and analyzed for

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9. Details of experimental design and procedures (continued)

elastase activity. Methods for assay of alveolar macrophages and leukocytes are outlined below. If the elastase activity of one or both of these cell sources is increased, the levels will be measured after cessation of exposure to NO_2 to determine if there is a return to normal.

It is noteworthy that Kleinerman (29,30) has reported the results of exposure of hamsters to NO_2 in concentrations of 45 to 55 ppm for 21 to 23 hours daily for ten weeks. In this species, enlargement of alveolar spaces, epithelial hyperplasia, and abundant inflammatory cells, predominantly neutrophiles and macrophages around respiratory bronchioles and alveolar ducts, were seen but no pulmonary emphysema. It is, therefore, pertinent to compare alveolar macrophage and polymorphonuclear leukocyte elastase activity in the rat and the hamster after similar exposure to NO_2.

As outlined, these experiments are concerned with the response of pulmonary macrophages and leukocytes to inhaled NO_2 with respect to the production of anatomical emphysema. However, the response with respect to cellular elastase activity is of importance whether or not emphysema is produced. The measurement of elastase activity is pertinent to the characterization of the injury produced in the lung by NO_2.

Studies requiring exposure of animals to nitrogen dioxide for prolonged periods to produce emphysema will be carried out in collaboration with Dr. Gustave Freeman of the Stanford Research Institute in Menlo Park, California, to house, expose and monitor the exposure of rats to desired concentrations of nitrogen dioxide as well as ozone and other atmospheric pollutants. Histological characteristics of exposure to nitrogen dioxide, ozone and the combination of the two, as well as the pattern and morphological features of pulmonary emphysema, resulting from exposure to NO_2 or the combination of NO_2 and O_3 have been extensively reported in publications by Dr. Freeman and his co-workers (27,28, 31-34).

Alveolar macrophages and polymorphonuclear leukocytes will be harvested from the experimental animals in Dr. Freeman's laboratory and the lysosomal enzyme preparation carried to the step where it may be held frozen at -20°C for prolonged periods without losing elastase activity. Specimens will be transported by air freight in the frozen state for assay for elastase activity in Dr. Mandl's laboratory.

The precise methods for generating NO_2 of desired concentration, for exposing rats to these concentrations over prolonged periods, have been described previously by Dr. Freeman and co-workers (27,28) and will not be recapitulated here. Dr. Freeman has indicated his willingness to collaborate in these studies and has so written in the attached letter.

As a later phase of this work, it will be possible to examine the effect on alveolar macrophage and polymorphonuclear leukocyte elastase of exposure to ozone in concentrations of from .5 ppm O_3 to .9 ppm O_3 or as a combination of NO_2 and O_3 (.9 ppm O_3 and .9 ppm NO_2) which has been shown to produce emphysema in a shorter interval than with NO_2 alone (29).

These studies of NO_2 and O_3 are of particular significance because NO_2 is produced by tobacco smoke and both are atmospheric pollutants.

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9. Details of experimental design and procedures (continued)

Morphological Studies

In each group of experimental animals in this portion of the study, one lung from representative animals will be inflation-fixed in formalin at 25 cm H_2O and sections will be stained and examined for emphysema by microscopic section and the emphysema graded by the point-counting method (35,36).

Parts of the remaining lung will also be prepared for electron microscopy. Alveolar lining cells, including the alveolar macrophages, will be examined by electron microscopy to determine if distinctive morphological alterations occur concomitant with change in alveolar macrophage elastase activity determined chemically. Studies of the ultrastructural characteristics of pulmonary interstitial elastin will also be carried out.

Dr. Joshua Fierer, who will conduct the morphological studies, has had experience over the past three years in the light and electron microscopy of both human and experimental emphysema in dogs and rats with particular interest in the electron microscopic appearance of pulmonary elastin and alveolar cell morphology.

2. Human Studies

In studies done thus far, in this Laboratory, it has been demonstrated that polymorphonuclear leukocytes of patients with obstructive lung disease have a statistically significantly higher activity of polymorphonuclear leukocyte lysosomal elastase than do normal control human subjects (22). However, no studies have been done to investigate the potentially pathogenetic role of alveolar macrophage elastase activity in chronic obstructive lung disease syndromes. Also, it has been demonstrated that there are statistically significant differences in alveolar macrophage esterase activity between normal subjects who are smokers and normal subjects who are non-smokers (16). It, therefore, becomes of some significance in understanding the pathogenetic mechanisms leading to alveolar damage in obstructive lung disease to determine if 1) alveolar macrophages in subjects with obstructive lung disease are significantly higher in elastase activity than normal subjects with an asymptomatic pulmonary status and normal pulmonary function, 2) whether patients with obstructive lung disease syndromes who are smokers and non-smokers have significantly different levels of alveolar macrophage elastase activity and 3) to compare the absolute levels of elastase activity of polymorphonuclear leukocyte lysosomes and of alveolar macrophage lysosomes with respect to elastase activity per cell and per microgram of lysosomal protein.

An added feature of this portion of the study will be an attempt to culture alveolar macrophages harvested from patients in vitro. It has been shown by Bennett (37) and by Soderland and Naum (38,39) that alveolar macrophages lavaged from mouse lung can be maintained in vitro in "standardized conditions" for periods of months. Identification of cells from the lung in culture was done by electron and light microscopy (39). A technique

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9. Details of experimental design and procedures (continued)

2. Human Studies (continued)

for separation of alveolar macrophages for cloning has been described and has utilized the special macrophage adhesiveness on plastic surfaces to eliminate other cell types (40,41). The capacity of alveolar macrophages to sustain elastase activity in tissue culture can be determined. Such in vitro preparations of cells will allow the investigation of various factors to ascertain the effect of such agents on the cells directly rather than through the in vivo respiratory system. Such agents are 1) tobacco smoke, 2) O_3 and NO_2, 3) hypoxic gas mixtures and 4) changes in pH of the media from acidosis to alkalosis around the norm of 7.40.

For these studies, subjects with obstructive lung disease syndromes are available in the in-patient and out-patient facilities of the Presbyterian, Harlem and Delafield Hospitals. The technique of pulmonary lavage in patients is outlined below.

All patients with obstructive lung disease who are part of this study will have a complete clinical and physiological evaluation. They will also have phenotyping of serum alpha$_1$-antitrypsin by acid starch gel crossed immunoelectrophoresis. The physiological evaluation will consist of measurements of total lung capacity and its components, arterial blood gas composition, single breath diffusing capacity for carbon monoxide and static recoil pressures of the lung at various lung volumes including total lung capacity. From the clinical appraisal, and these physiological measurements, an attempt will be made to distinguish those patients in whom pulmonary emphysema is a predominant component of their obstructive airway disease in an attempt to define correlations between the occurrence of emphysema and quantitative increase in elastase activity in alveolar macrophages and polymorphonuclear leukocytes.

Alveolar macrophages will be obtained in the course of routine bronchoscopies by fiberoptic bronchoscopy for clinical purposes. To obtain alveolar macrophages from normal human subjects, patients coming to bronchoscopy because of isolated small pulmonary infiltrates on chest x-ray without symptoms of pulmonary dysfunction and with normal pulmonary function by physiological testing will be selected. Such patients are now available in the Pulmonary Disease Section at Presbyterian and Harlem Hospitals. Similarly, patients with obstructive lung disease in various degrees of severity come to bronchoscopy in the course of their disease because of the appearance of new pulmonary densities which require visualization, biopsy or brush biopsy. With the consent of the patient, lavage of aliquots of 50 ml of isotonic saline for a total of 200 to 300 ml of fluid warmed to 37°C, will be used for lavage of alveolar macrophages.

In the technique of pulmonary lavage, described by Finley et al. (42), the Metras balloon-tipped catheter was used to instill and withdraw smaller aliquots of isotonic saline, warmed to 37°C, from certain regions of the lung. In normal subjects, good recoveries of the instilled saline were achieved, whereas in patients with obstructive lung disease, recoveries of fluid were poor because of trapping of fluid in emphysematous areas. The use of the fiber-optic flexible bronchoscope and the introduction of small diameter catheters through the

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9. Details of experimental design and procedures (continued)

2. Human Studies (continued)

bronchoscope to much more distal airways than can be achieved by the Metras catheter shoul.d allow recovery of adequate volumes of fluid to promote satisfactory alveolar macrophage yields. If necessary, the patient can change position on the fluoroscopy table to assist drainage of the instilled fluid.

The technique for obtaining and assaying polymorphonuclear leukocyte lysosomal elastase activity is also outlined below. This technique requires approximately 60 ml of venous blood. No difficulty has been experienced in obtaining this volume of blood from patients thus far even for repeated determinations.

The principal investigator at present directs a Program Project from the National Institutes of Health entitled "Chemical Predispositions to Pulmonary Injury." This study includes in large part work on the connective tissue composition of the lung in chronic obstructive lung disease in patients and in experimental emphysema induced in animals by proteolytic enzymes and also in diffuse fibrotic reactions of the lung. These studies are focussed on the elastin, collagen and glycosaminoglycans of the lung in the normal state and in disease and the amino acid composition of these components. The studies outlined in the proposal now being submitted are not being done under the NIH supported work and are not a planned part of that project.

In this Program, so far, tissue culture techniques have been employed for various parts of the study and are carried out by Dr. Mary Parshley of the Department of Pathology here. Cell lines of human and rat lung endothelium and fibroblasts have been established in her laboratory and the techniques now in use would be adapted to growing cell lines of alveolar macrophages.

Techniques for Measurement of Elastase Activity of Alveolar Macrophagesand Polymorphonuclear Leukocytes

The technique for the measurement of polymorphonuclear leukocyte elastase activity has been standardized in this laboratory over the past 2 years. The method involves the drawing of 60 ml of venous blood anticoagulated with citrate and is well tolerated by patients. Blood is mixed with 3% dextran in isotonic NaCl and erythrocytes are allowed to settle. Leukocytes are obtained by centrifugation of the supernates washed in 0.34 M sucrose and a suspension is disrupted mechanically by a vacuum suction through a fine wire screen. The diluted homogenate is then subjected to differential centrifugation and the granular fraction which contains elastolytic activity resuspended in 0.15 M phosphate saline buffer pH 7 and disrupted by freeze-thawing. The elastolytic activity is then measured by an assay developed in our own laboratory using a soluble elastin substrate (43).

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9. Details of experimental design and procedures (continued)

Techniques for Measurement of Elastase Activity of Alveolar Macrophagesand Polymorphonuclear Leukocytes (continued)

Several aliquots of 50 ml for a total of 250-300 ml will be used to obtain an adequate number of alveolar macrophages for the measurement of elastolytic activity. In all cases the lavage fluids will be filtered first through gauze, then centrifuged for 30 minutes at 17,000 x g to sediment the macrophage granules. This is a higher speed than that used for leukocyte granule separation and has been recommended by Janoff, Rosenberg and Galdston (15). Total cell counts, differential counts, etc. will have to be completed before this step. All subsequent steps will follow the procedure successfully applied in our laboratory to the isolation of leukocyte elastase. Essentially this involves washing twice with cold 0.34 M sucrose solution, followed by mechanical cell disruption of the sucrose suspension by vacuum suction through a fine wire screen. The granular fraction is then separated by differential centrifugation, first at 900 x g to remove unbroken cells and debris leaving the granules in solution, then at 20,000 x g to sediment the granules from the resuspended supernatant. The precipitate in the form of a small greenish dot contains all the elastolytic activity and is carefully washed and suspended in cold 0.15 M phosphate saline buffer pH 7.0. It is disrupted by 7 cycles of freeze-thawing to liberate the enzyme and centrifuged again at 20,000 x g to remove all debris leaving the lysosomal extract in solution. The material will be stored at this stage at -70°C or, if sufficient material is available, subjected to additional purification by affinity chromatography on CNBr activated Sepharose 4B coupled to soluble elastin. Elastase activity will be assayed against solubilized elastin by the method of Keller and Mandl (43) or against the sensitive and specific synthetic substrate succinyl alanine alanine alanine p-nitroanilide introduced by Bieth, Spiess and Wermuth (44). Elastolytic activity is expressed in μ units per μ gram protein determined by the assay of Lowry et al. (45). Since the number of cells being assayed is also known, the results will also be analyzed in terms of elastase activity per unit cell.

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32. Freeman, G., Furiosi, N.J. and Haydon, G.B.: Effects of continuous exposure to 0.8 ppm NO_2 on respiration of rats. Arch. Environ. Health, 13: 454, 1966.

33. Freeman, G., Crane, S.C., Stephens, R.J. and Furiosi, N.J.: Pathogenesis of the nitrogen dioxide-induced lesion in the rat lung. A review and presentation of new observations. Am. Rev. Resp. Dis., 98: 429, 1968.

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41. Leuchtenberger, C. and Leuchtenberger, R.: Cytological and cytochemical effects on primary mouse kidney tissue and lung organ cultures after exposure to whole fresh smoke and its gas phase from unfiltered, charcoal filtered and cigar tobacco cigarettes. Cancer Res., 29: 862, 1969.

42. Finley, T.N., Swenson, E.W., Curran, M.S., Hurer, G.L. and Ladman, A.T.: Bronchopulmonary lavage in normal subjects and patients with obstructive lung disease. Ann. Int. Med., 66: 651, 1967.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Laboratories of Dr. Gustave Freeman
Stanford Research Institute
Menlo Park, California

11. Additional facilities required:

none

12. Biographical sketches of investigator(s) and other professional personnel (append):

see attached

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available):

see attached

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12. Biographical sketches of investigators :

Gerard M. Turino, M.D., is Professor of Medicine, Columbia University College of Physicians and Surgeons and Director of the Cardiovascular Laboratory of the Presbyterian Hospital. REDACTED graduated from Princeton University (A.B.) in R and from the Columbia University College of Physicians and Surgeons in R Medical Internship and one year of residency in medicine were completed at the Columbia University Division at Bellevue Hospital 1948-1950 and one year of assistant residency in medicine at the Yale-New Haven Community Hospital in 1950-1951. In 1953-1954 he was chief resident in medicine at the Columbia University Division at Bellevue Hospital. From 1951-1953 he was a Captain United States Air Force Medical Corps and served as a professional associate in the Division of Medical Services of the National Research Council, Washington, D. C. He has been on the faculty of Columbia University College of Physicians and Surgeons since 1955. From 1956-1961, he was a senior Fellow of the New York Heart Association and from 1961-1971, a career investigator of the Health Research Council of the City of New York. His research interests during this time have been in cardiorespiratory physiology and diseases of the cardiorespiratory system. 40% of his time is devoted to teaching, clinical work and administration and 60% to research. He is a member of the Editorial Boards of the American Review of Respiratory Disease and the journal, Respiration. He was a member of the Task Force on Obstructive Lung Disease of the National Heart and Lung Institute and Chairman of its subcommittee on "Chemical Structure and Function of the Lung". He is a member of the

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Pertinent Publications:

Turino, G.M., Goldring, R.M. and Heinemann, H.O.: Water Electrolytes and Acid-Base Relationships in Cor Pulmonale. Prog. in Cardiovascular Diseases, 12:167, 1971.

Turino, G.M., Lourenco, R.V. and McCracken, G.H.: Role of Connective Tissue in Large Pulmonary Airways. J. Appl. Physiol., 25:645, 1968.

Turino, G.M., Senior, R.M., Garg, B.D., Keller, S., Levi, M.M., Mandl, I.: Serum Elastase Inhibitor Deficiency and Alpha$_1$-Antitrypsin Deficiency in Patients with Obstructive Emphysema, Science 165:709, 1969.

Turino, G.M. and Lourenco, R.V.: The Connective Tissue Basis of Pulmonary Mechanics. In: International Symposium on Proteolysis and Pulmonary Emphysema. Academic Press, 1972. p.509.

Turino, G.M.: Proteolytic Mechanisms and Pulmonary Emphysema. Bull. Physio-Path. Resp. 7:719, 1971.

Turino, G.M., Rodriguez, J.S., Greenbaum, L.M. and Mandl, I.: Mechanisms of Pulmonary Injury. Am. J. Med. 57:493-505, 1974.

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12. Biographical sketches of investigators:

Joshua A. Fierer, M.D., Director of Immunopathology, Assistant Professor of Pathology, Columbia University College of Physicians & Surgeons. REDACTED

REDACTED graduated from Alfred University, Alfred, New York (B.A.) in R and from the State University of New York Downstate Medical Center (M.D.) in R. He was an intern in Surgery in 1963, resident in Surgery in 1964, and Assistant in Surgery in 1965 at the University of Rochester School of Medicine and Strong Memorial Hospital, Rochester, New York. From 1965-1967 he served in the United States Air Force Medical Corps at the School of Aerospace Medicine, Texas, and as Chief of Aeromedical Services, Stewart AFB, New York. He completed a residency in Pathology at Columbia Presbyterian Medical Center, New York, and joined Columbia University College of Physicians and Surgeons as an Instructor in Pathology in 1969. During 1969/70 he was a Guest Investigator in Dr. H. Kunkel's Laboratory at Rockefeller University and since 1970 he has been Assistant Professor of Pathology at Columbia University College of Physicians and Surgeons and Director of Microbiology at The Francis Delafield Hospital Division. In 1972 he received an additional appointment as Assistant Attending Pathologist, Presbyterian Hospital, New York and Director of Immunopathology, Columbia University College of Physicians and Surgeons. He is a member of the

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Pertinent Publications:

Buda, J.A., Suciu-Foca N., Fierer, J.A. and Geering, G.: Induction of Runt Disease in Newborn Rats by Prolonged Antigenic Stimulation with Acellular Tissue Extracts. Transplantation 13:332, 1972.

Werner, S.C., Wegelius, O., Fierer, J.A. and Hsu, K.C.: Immunoglobulins (E,M,G) and Complement in the Connective Tissues of the Thyroid in Graves' Disease. N.E.J.M. 287:421, 1972.

Fierer, J.A., LoGerfo, P. and Bennett, S.: Immunofluorescent Identification of a Tumor-Associated Antigen in Neoplastic and Non-neoplastic Human Tissues. Fed. Proc. 31:661, 1972.

Fierer, J.A., Klavins, J.V., Weiss, M., Mesa-Tejada, R., Berkman, J.I. and Rollwagen, F.: Oncofetal Antigens Extracted from Human Embryonic Tissues. Am. J. Path. 70:11a, 1973.

Mandl, I., Keller, S., Fierer, J.A., and Evans, H.E.: The Role of Proteolytic Enzyme Inhibitors and Connective Tissue Proteins in the Maturation of the Lung. In Respiratory Distress Syndrome. Claude A. Villee, Dorothy Villee, and James Zuckerman eds. p.99, Academic Press, 1973.

Fierer, J.A., Mao, T.S.S. and Appleton, R.: Isolation of a New Oncofetal Antigen from Human Malignant Neoplasms. Am. J. Path. 74:62a, 1974.

Fierer, J.A., Mandl, I., and Evans, H.E.: Alpha$_1$-Antitrypsin in the Lungs of Newborns with Respiratory Distress Syndrome. J. Ped. 85:698, 1974.

Blackwood, C.E., Moret, J.E., Keller, S., Fierer, J.A. and Mandl, I.: Alpha$_1$-Antitrypsin Concentration in Serum of Laboratory Animals. Changes Following Proteolytic Enzyme Injection. J. Lab. Clin. Med. 84:813, 1974.

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12. Biographical sketches of investigators:

Ines Mandl, Ph. D., Associate Professor of Reproductive Biochemistry, R REDACTED received her early education in Austria and at the National University of Ireland, in Cork, Eire, and her M.S. and Ph.D. degrees at the Polytechnic Institute of Brooklyn in R and R respectively. From 1945-1949, she was assistant and coworker of the late Professor Carl Neuberg at New York University and at Interchemical Corporation. Since 1949, she has been at Columbia University College of Physicians and Surgeons, first as chief chemist on an Army sponsored project in the Department of Surgery with Dr. E. L. Howes and Dr. John D. McLennan. From 1956 to 1973 she has been Assistant Professor of Biochemistry assigned first to the Department of Microbiology and, since 1959, to the Department of Obstetrics and Gynecology in charge of the Gynecology Research Laboratories at the Francis Delafield Hospital Division. In 1972 she was awarded a Distinguished Alumnus Award by the Polytechnic Institute of Brooklyn. She is a member of the

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She is also Editor-in-Chief of Connective Tissue Research, An International Journal.

Pertinent Publications:

Manahan, J. and Mandl, I.: Primary Structure of Insoluble Tendon Collagen. I. N- and C- Terminal Positional Analysis of Collagenolytic Breakdown Products of Native Collagen. Arch. Biochem. Biophys. 128:6-18, 1968.

Turino, G.M., Senior, R.M., Garg, B.D., Keller, S., Levi, M.M., Mandl, I.: Serum Elastase Inhibitor Deficiency and Alpha$_1$-Antitrypsin Deficiency in Patients with Obstructive Emphysema, Science 165:709-711, 1969.

Keller, S., Levi, M. and Mandl, I.: Antigenicity and Chemical Composition of an Enzymatic Digest of Elastin. Arch. Biochem. Biophys. 132:565-572, 1969.

Keller, S. and Mandl, I.: Qualitative Differences Between Normal and Emphysematous Human Lung Elastin. In: Pulmonary Emphysema and Proteolysis. Ed. by C. Mittman, Academic Press, pp. 251-259, 1972.

Blackwood, G.C., Hosannah, Y., Perman, E., Keller, S. and Mandl, I.: Experimental Emphysema in Rats: Elastolytic Titer of Inducing Enzyme as Determinant of the Response. Proc. Soc. Exp. Biol. Med. 144:450-434, 1973.

Keller, S. and Mandl, I.: Elastin Peptides: The Components of a Partial Alkaline Hydrolyzate of Elastin. Conn. Tissue Res. 2:49-56, 1973.

Evans, H.E., Keller, S. and Mandl, I.: Lung Tissue Elastin Composition in Newborn Infants with the Respiratory Distress Syndrome and Other Diseases. J. Clin. Invest. 54:213-217, 1974.

Turino, G.M., Rodriguez, J.S., Greenbaum, L.M. and Mandl, I.: Mechanisms of Pulmonary Injury. Am. J. Med. 57:493-505, 1974.

Parshley, M.S., Sampson, P., Mandl, I. and Turino, G.M.: Production of Acidic Glycosaminoglycans by Normal Rat Lung in Tissue Culture. Abstr. J. Cell Biol. 63:258, 1974.

Mandl, I., Darnule, T., Keller, S. and Fierer, J.A.: Changes With Age in the Microfibrillar Component of Lung Elastin. Abstr. 10th International Congr. Gerontol. 1975.

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12. *Biographical sketches of investigators:*

Mary S. Parshley, Ph.D. REDACTED Research Associate in Pathology and Clinical Fellow in Medicine She obtained her A.B. from Smith College in R and M.A. and Ph.D. degrees in anatomy from the University of Pennsylvania in R and R respectively. She was a Finney-Howell Postdoctoral Fellow in Anatomy at the University of Pennsylvania from 1938-1939, following which she joined the Dept. of Pathology of Columbia University College of Physicians and Surgeons as a Research Assistant from 1940-1945, advancing to Research Associate 1945-1949. In 1949 she became a Research Associate in Surgery and in 1962 Asst. Professor of Anatomy. She assumed her current position in 1973. She is a charter member of REDACTED served as an officer of this Association. She is also a member REDACTED REDACTED REDACTED

Pertinent Publications:

Parshley, M.S.: The tissue culture of adult tissues. Chap. 17 in the "Transplantation of Tissues", Vol. II, Lyndon Peer, ed., Williams and Wilkins Co., Baltimore, pp. 593-633, 1959.

Simms, M.S. and Parshley, M.S.: The effect of proteins and amino acids on the growth of adult tissue in vitro. Chap. 7 in "Protein and Amino Acid Nutrition", A. Albanese, ed., Academic Press, N.Y., pp. 143-195, 1959.

Parshley, M.S.: Effect of inhibitors from adult connective tissue on growth of a series of human tumors in vitro. Cancer Res. 25:387-399, 1965.

Parshley, M.S., and Mandl, I.: Inhibition of malignant cells in vitro by a component of normal adult connective tissue. Nature, 203:800, Nov., 1965.

Levi, M.M., Parshley, M.S., and Mandl, I.: Antigenicity of papillary serous cystadenocarcinoma tissue culture cells. Am. J. Obst. and Gyn., 102: 433, 1968.

Martorelli, B., Jr., Parshley, M.S., and Moore, J.G.: Effects of chemotherapeutic agents on two lines of human breast carcinoma cells. Surgery, Gynecology & Obstetrics 127:1006, 1969.

Einbinder, J., Parshley, M.S., Walzer, R. and Sanders, S.L.: Effect of cantharidin on malignant epithelium in tissue culture. J. Invest. Derm. 52:291, 1969.

Parshley, M.S., Sampson, P., Mandl, I. and Turino, G.M.: Production of acidic glycosaminoglycans by normal rat lung in tissue culture. J. Cell Biol. 63:258, 1974.

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14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Salary	Obligatory Fringe Amount	Total
Gerard M. Turino, M.D.	20			
Joshua A. Fierer, M.D.	20			
Ines Mandl, Ph.D.	10	REDACTED		
Mary Parshley, Ph.D.	10			
Technical				
Ja-Shein Lin, B.S., Sr. Technician	100			
Asha Darnule, M.S., Technician	100	REDACTED		
John Moret, Animal Technician	100			
Mary Lynch, Glassware Washer	33 1/3			
Sub-Total for A		R		
B. Consumable supplies (by major categories)				
Glassware and minor equipment				3,500.
Chemicals				3,500.
Tissue Culture Media and Supplies				2,500.
EM and Photographic Supplies				2,500.
Animals				4,000.
Sub-Total for B		16,000.		
C. Other expenses (itemize)				
Animal Care				2,500
Repair and Maintenance				1,500
Publication Expenses				750
Travel				1,000
Sub-Total for C		5,750.		
Running Total of A + B + C		84,968.		
D. Permanent equipment (itemize)				
Smoking Machine				3,000
Sorvall Centrifuge RC-5 and rotors				4,950
Sub-Total for D		7,950.		
E. Indirect costs (15% of A+B+C)		12,745.		
Total request		105,663.		

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R	5,120.	5,130.	- 0 -	13,192.	101,140.
Year 3	R	5,330.	5,540.	- 0 -	14,247.	109,230.

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Chemical Predispositions to Lung Injury.	National Heart and Lung Institute - HL 15832	$1,321,303. (5 years)	May 1, 1973 to April 30, 1978.
Chemical Composition of Elastin, Collagen and Glycosaminoglycans of Lungs in Diffuse Interstitial Fibrosis from Sarcoid and Non-Sarcoid Pulmonary Fibrotic Reactions.	New York Lung Association	$23,000. (1 year)	April 1, 1975 to March 31, 1976.

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
None			

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Gerard M. Turino, M.D.

Signature [signature] Date 7/1/75

Telephone REDACTED
Area Code Number Extension

Checks payable to

Trustees of Columbia University

Mailing address for checks

c/o Frederick B. Putney, Ph.D.
630 West 168th Street
New York, New York 10032

Responsible officer of institution

Typed Name Frederick B. Putney, Ph.D.

Title Assistant Vice President for Health Sciences Administration

Signature [signature] Date 9/15/75

Telephone REDACTED
Area Code Number Extension

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